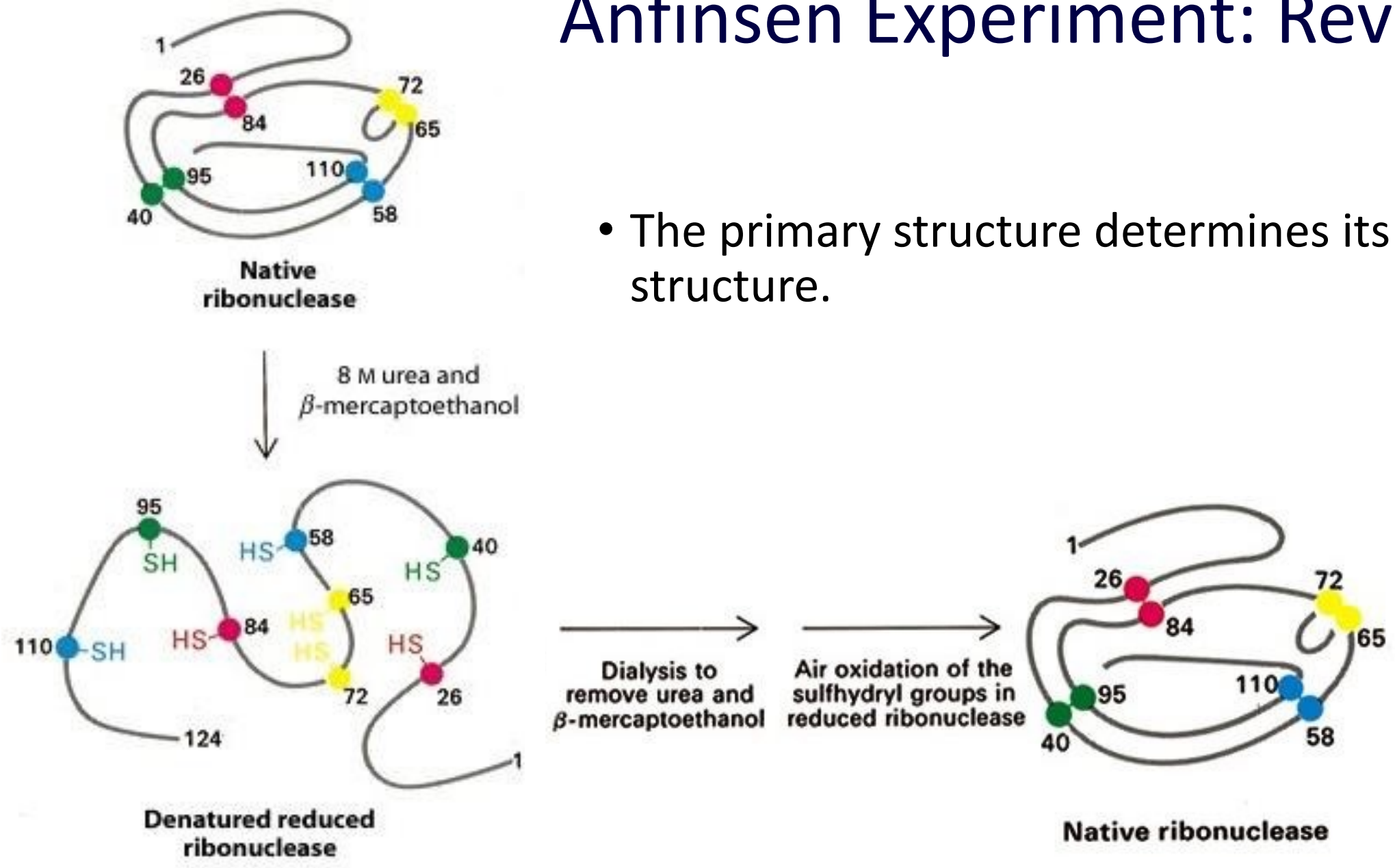
Lecture 7&8 -2

Bio312

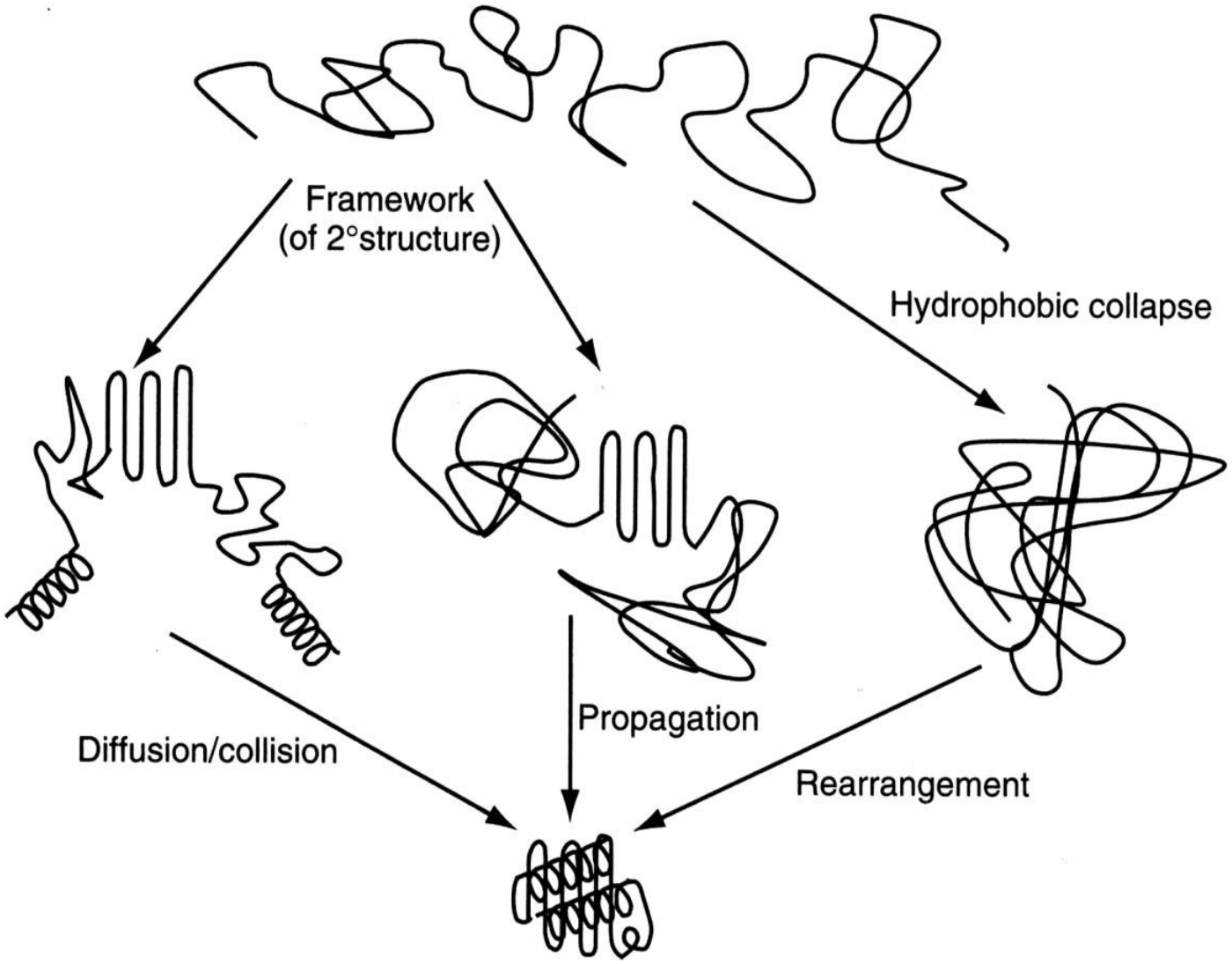
Instructor: Dr. Lanlan Han
E-mail: Lanlan.Han@xjtlu.edu.cn

Anfinsen Experiment: Review

- The primary structure determines its tertiary structure.



Three Classic Models of Protein Folding: Review



The Folding Funnel: Review

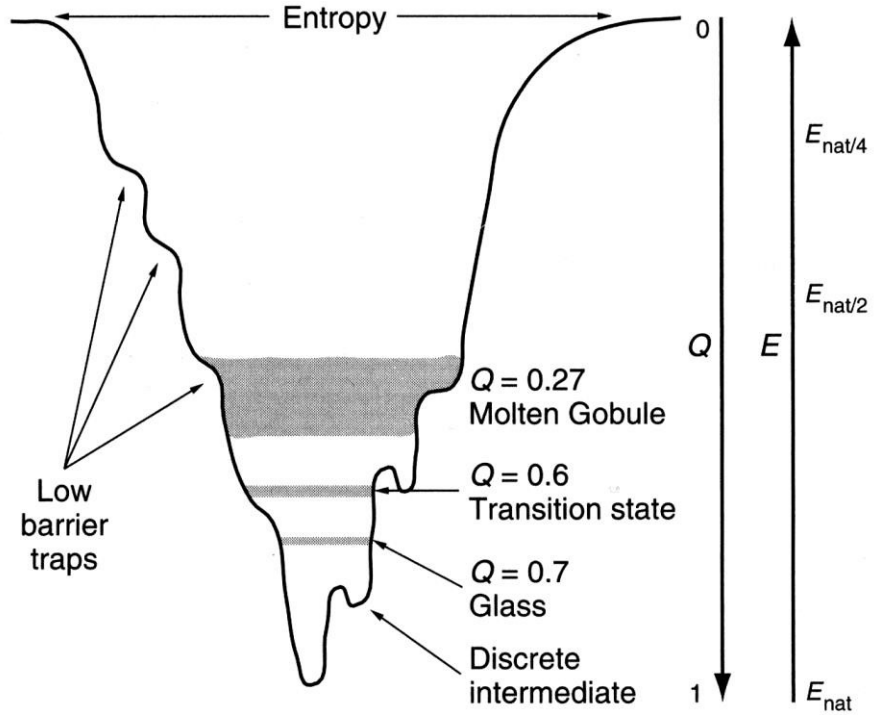
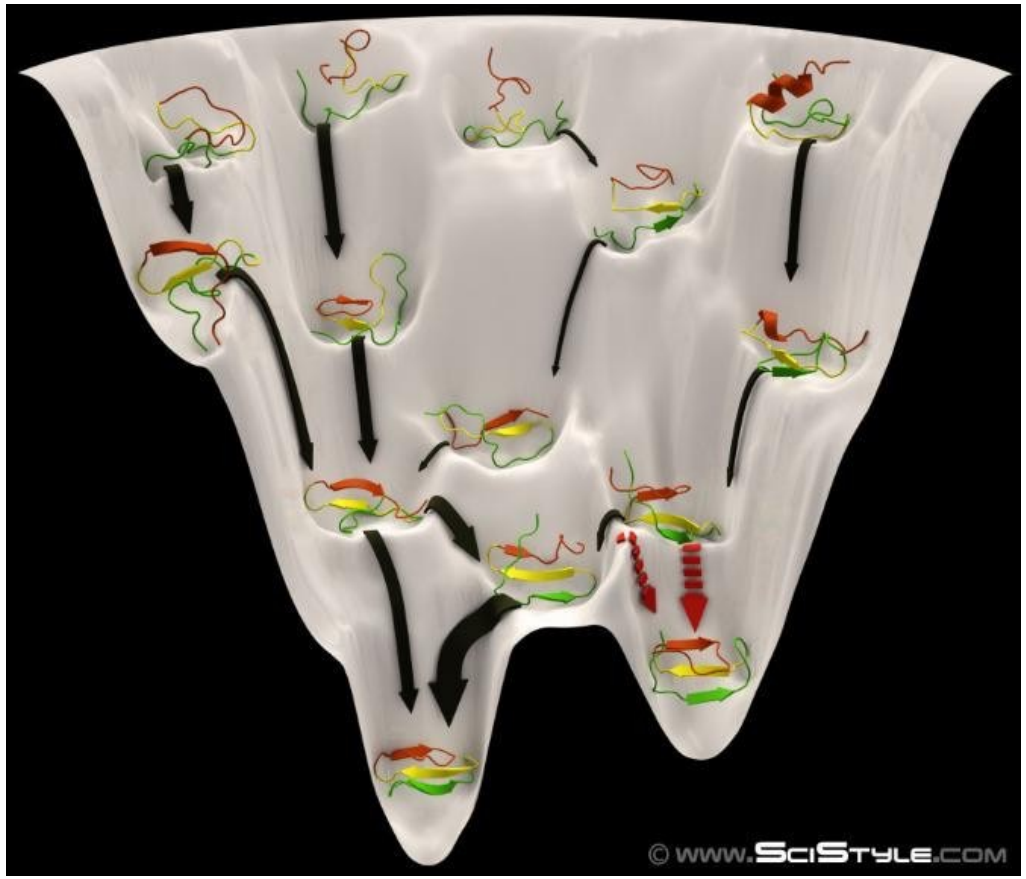


Figure 19.16 Cross section through a folding funnel. *E* corresponds to free energy. [Courtesy of P. G. Wolynes]

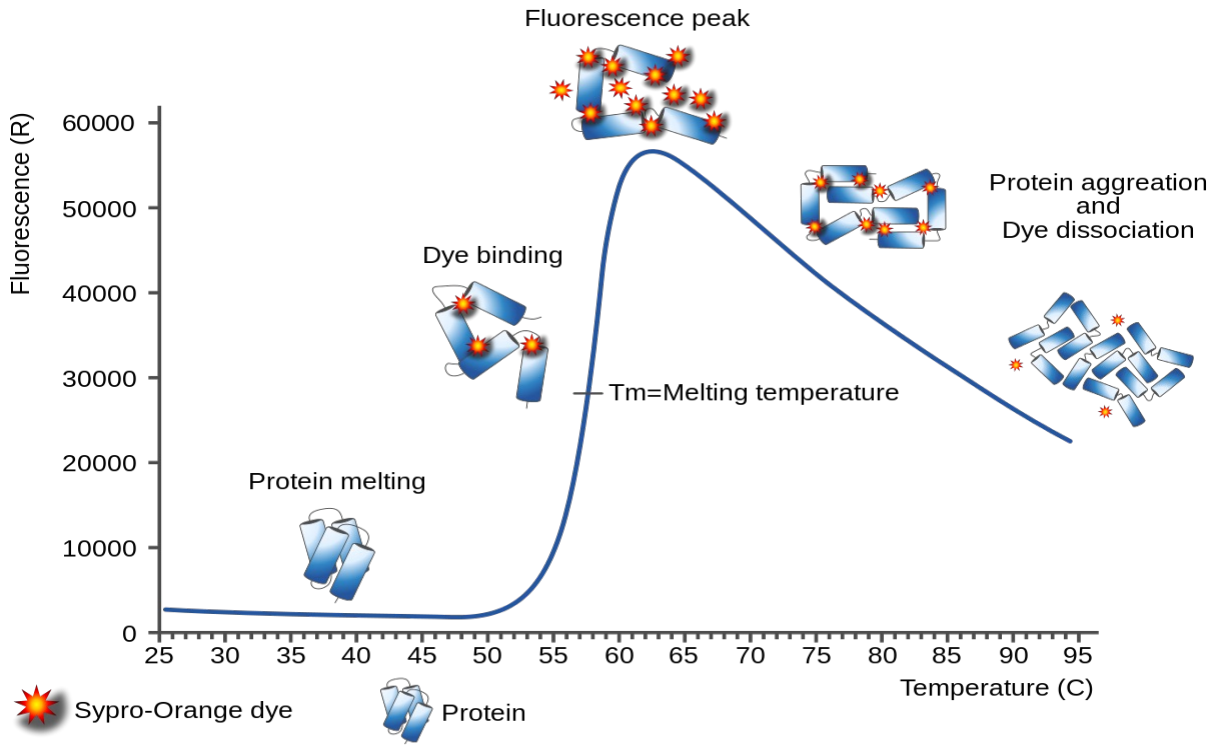
- The funnel is a collections of geometrically similar structure to native proteins.



Protein's native state is its free energy minimum

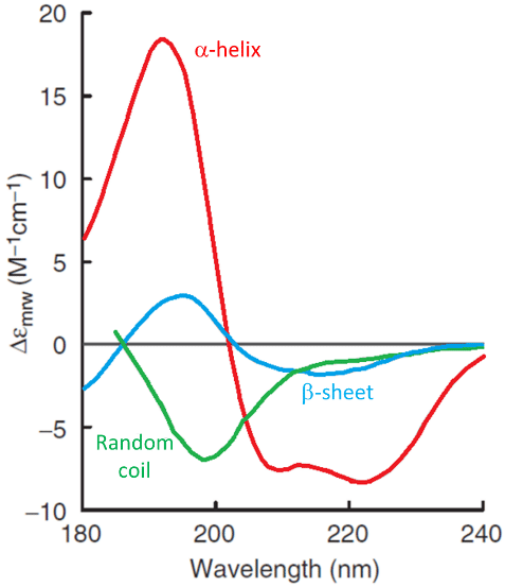
Thermal Shift Assay: Review

- *Principle*: Protein hydrophobic core is buried. When the protein solution is heated up, the protein is denatured, and hydrophobic region is exposed to SYPRO™ orange fluorescent dye. The fluorescent signal can be determined in real time instrument.

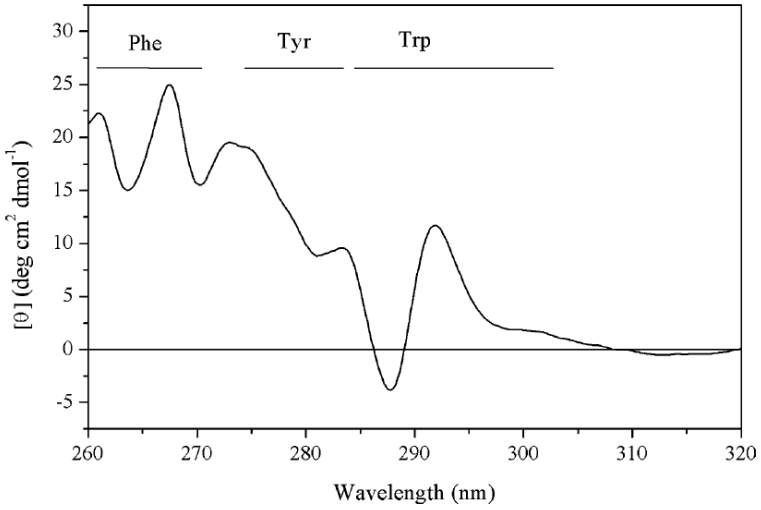


Circular Dichroism (CD) for Protein Structure: Review

- Different wavelength probe different levels of structure:
  1. **Far UV** (smaller wavelengths, 190-250 nm)
     - ➢ probing different secondary structures in a protein
  2. **Near UV** (longer wavelengths, 260-320 nm)
     - ➢ Probing aromatic amino acids--> tertiary structure change information

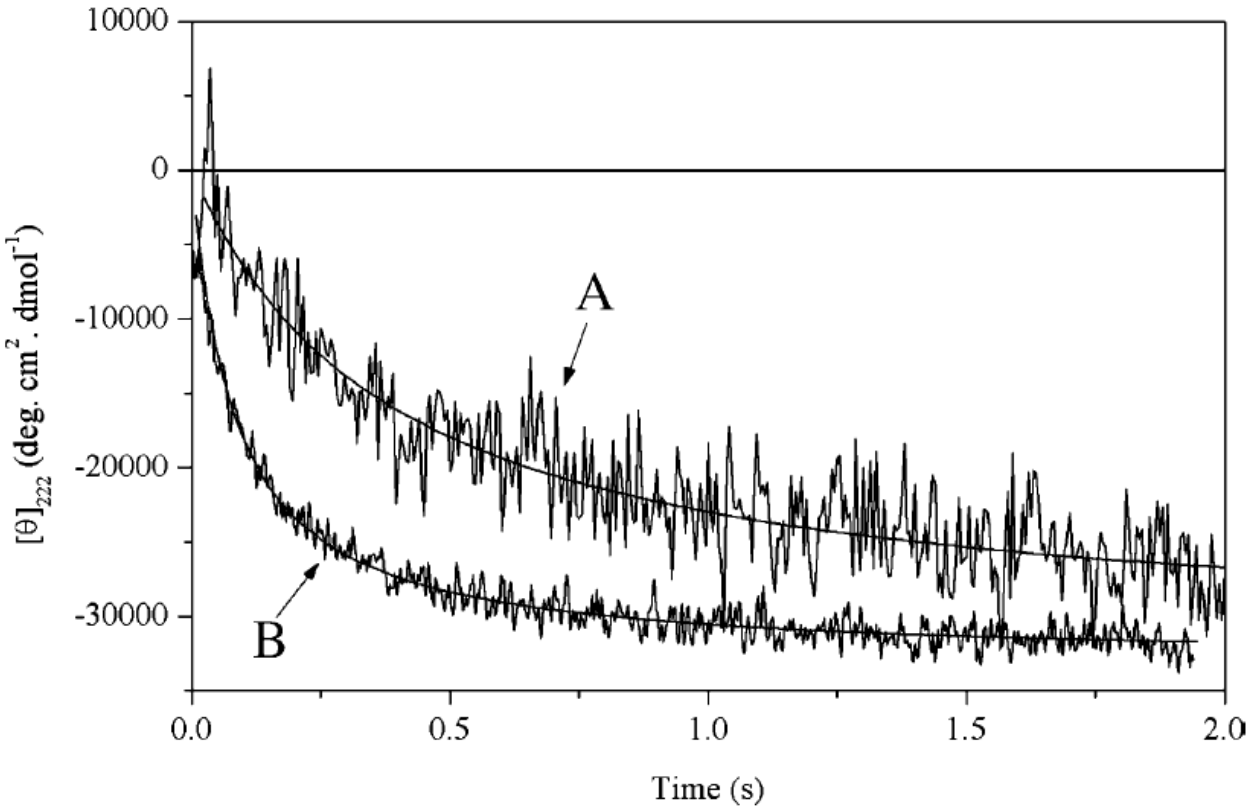


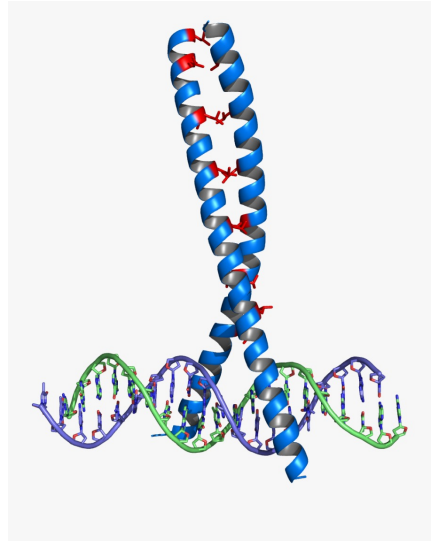
- α helix : negative bands at 208 and 222 nm and positive band at 193 nm
- β sheet : negative band at 218 nm and positive band at 195 nm
- Random coil: negative band at 195 nm and low ellipticity above 210 nm



4. Structural Stability (Folding or Unfolding)

The refolding of a leucine zipper peptide monitored by stopped flow CD at 222 nm.





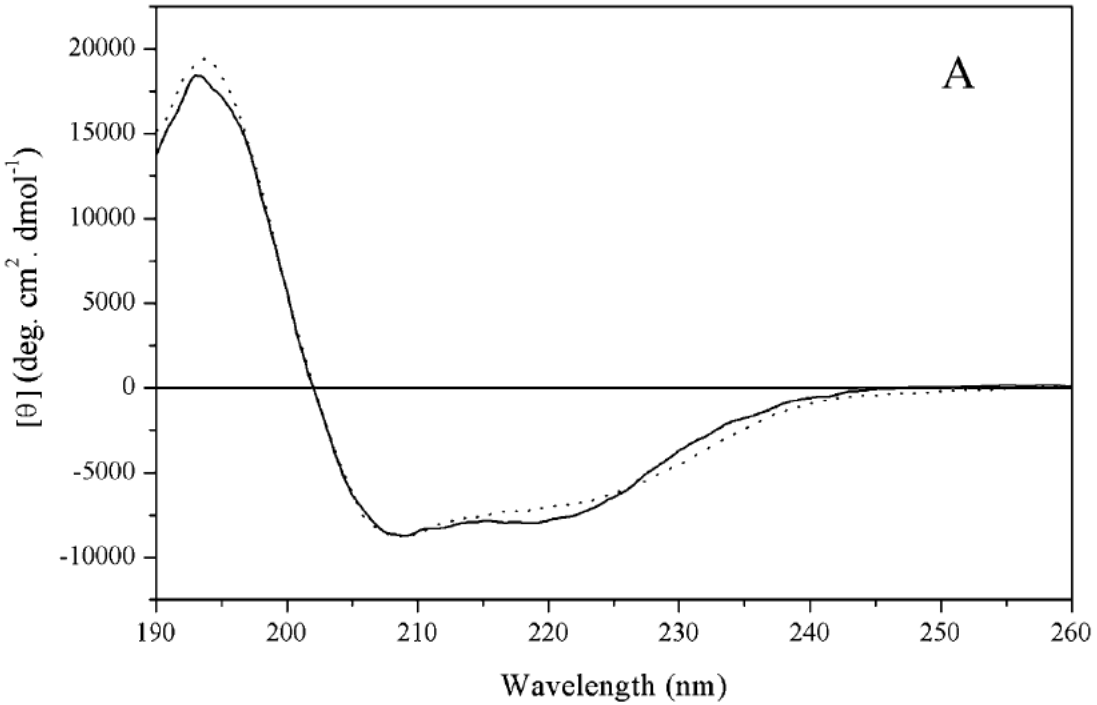
33-residue peptide at concentrations of 6 µM (A) and 26 µM (B) was refolded after denaturation in GdmCl.

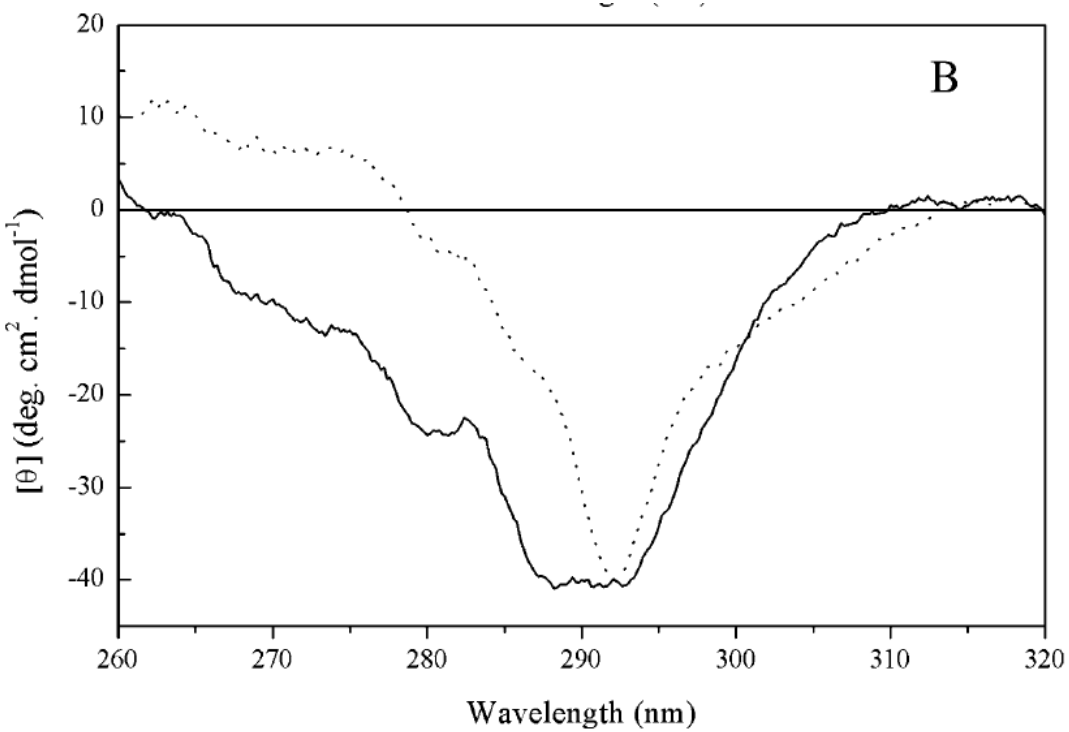
Zitzewitz JA, Bilsel O, Luo J, Jones BE, Matthews CR. Biochemistry. 1995 Oct 3;34(39):12812-9. doi: 10.1021/bi00039a042. PMID: 7548036.

5. Conformational Changes in Protein-Ligand Binding$_1$

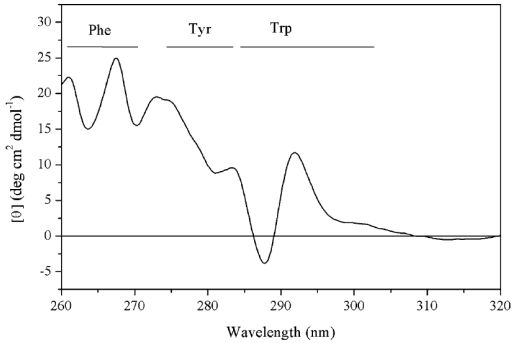
molybdate-sensing protein ModE (solid line)

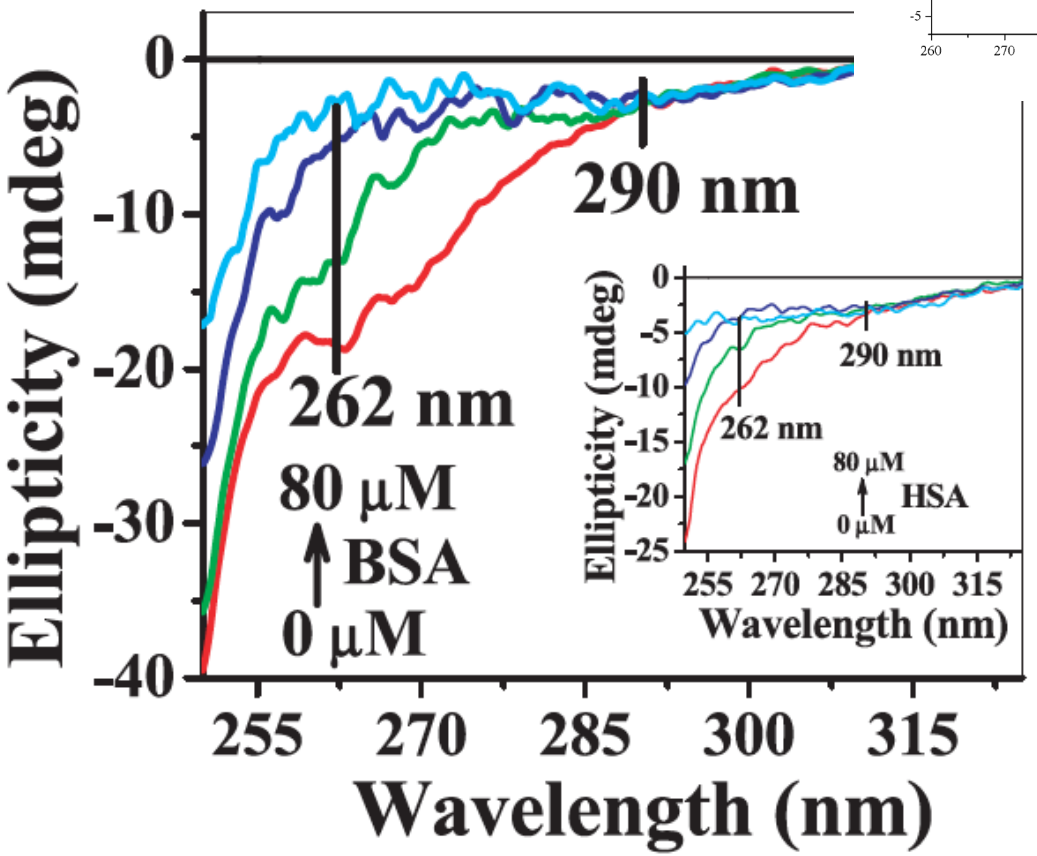
molybdate-sensing protein ModE + 1 mM molybdate (dotted line)

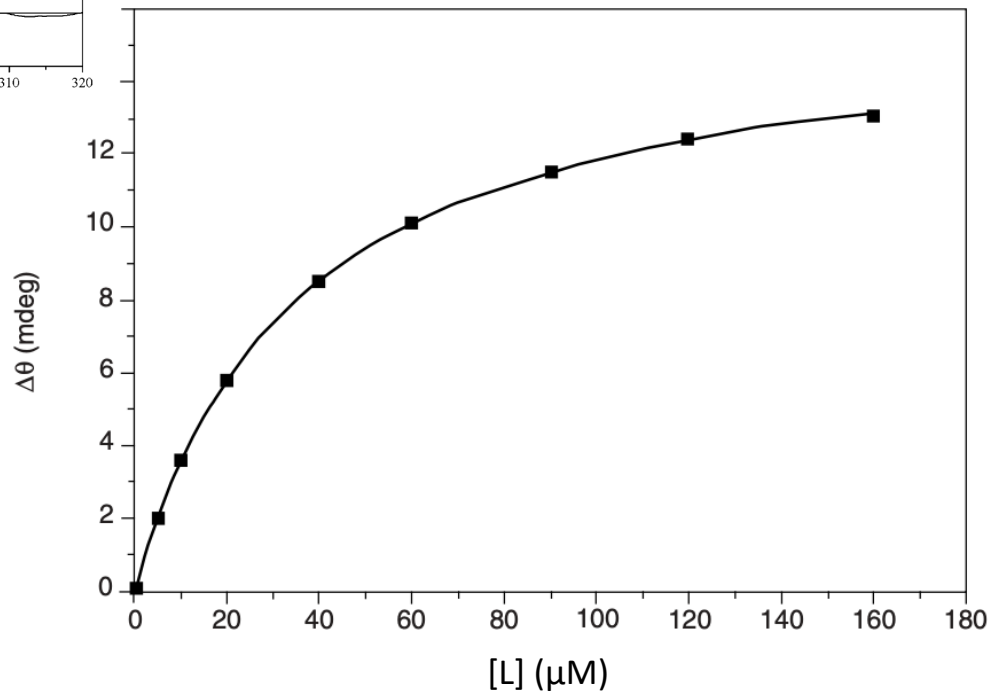




5. Conformational Changes in Protein-Ligand Binding$_2$





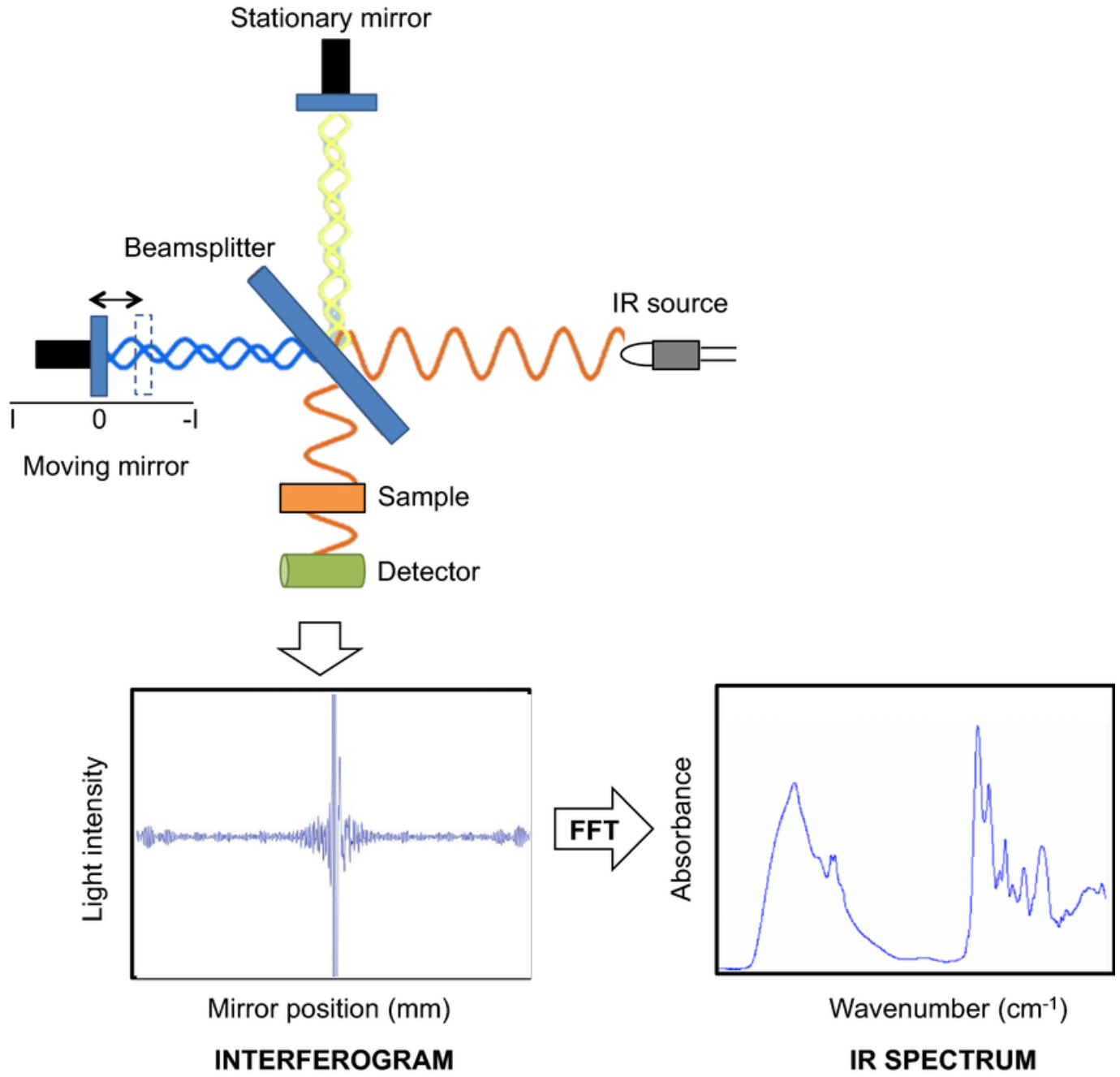


$$\Delta\theta = \Delta\theta_{max}\left([L_t]/(K_d + [L_t])\right)$$

Infrared (IR) Spectroscopy

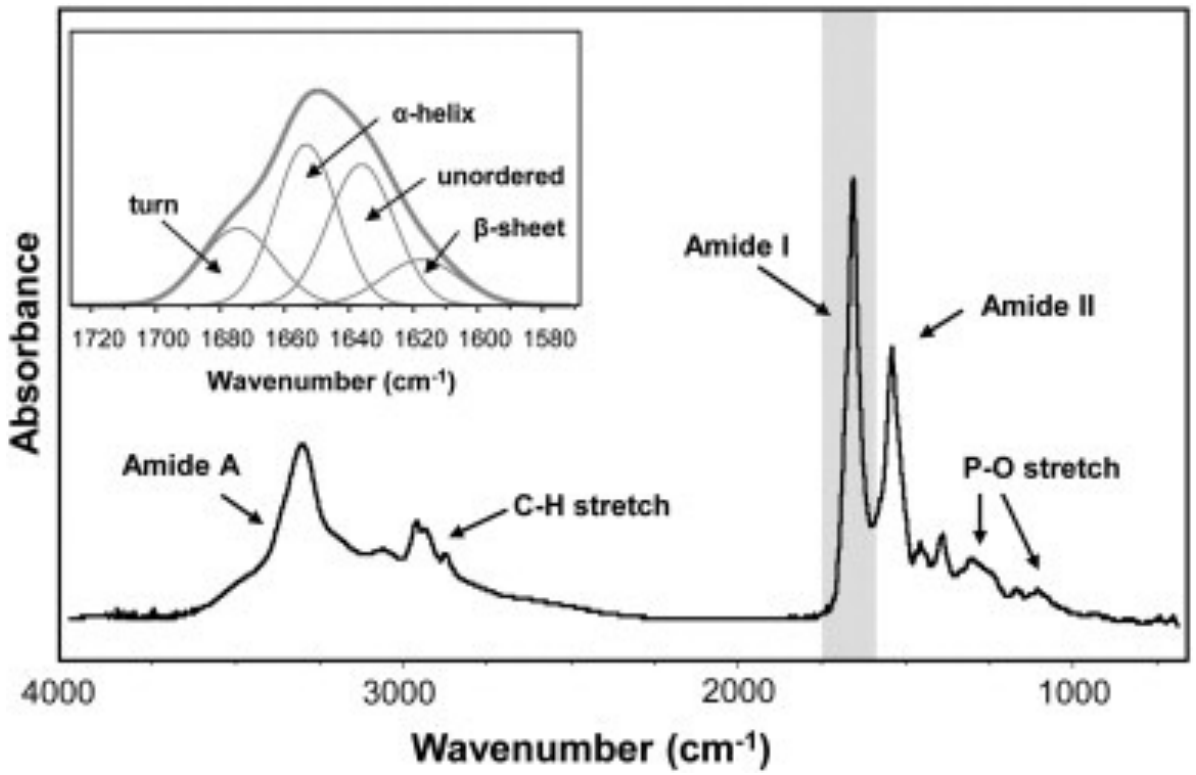
- IR spectroscopy studies the interaction between matter and infrared radiation.
- IR radiation is absorbed at characteristic wavelengths (wave number, $1/\lambda$) by different groups.
  - C=O groups absorb in the IR at 1550-1750 cm^{-1}
- IR is not a great technique for studying proteins, because water absorbs IR radiation strongly in regions that overlap the protein absorbance bands.

Fourier Transform IR (FTIR)



Campanella, Beatrice & Palleschi, Vincenzo & Legnaioli, S.. (2021). Introduction to vibrational spectroscopies. ChemTexts. 7. 10.1007/s40828-020-00129-4.

FTIR Spectra



- FTIR can be used to determine the amounts of each kind of 2° structure and the conformation analysis.
- Amide I and amide II band shape is very characteristic for a protein's 2° structure.

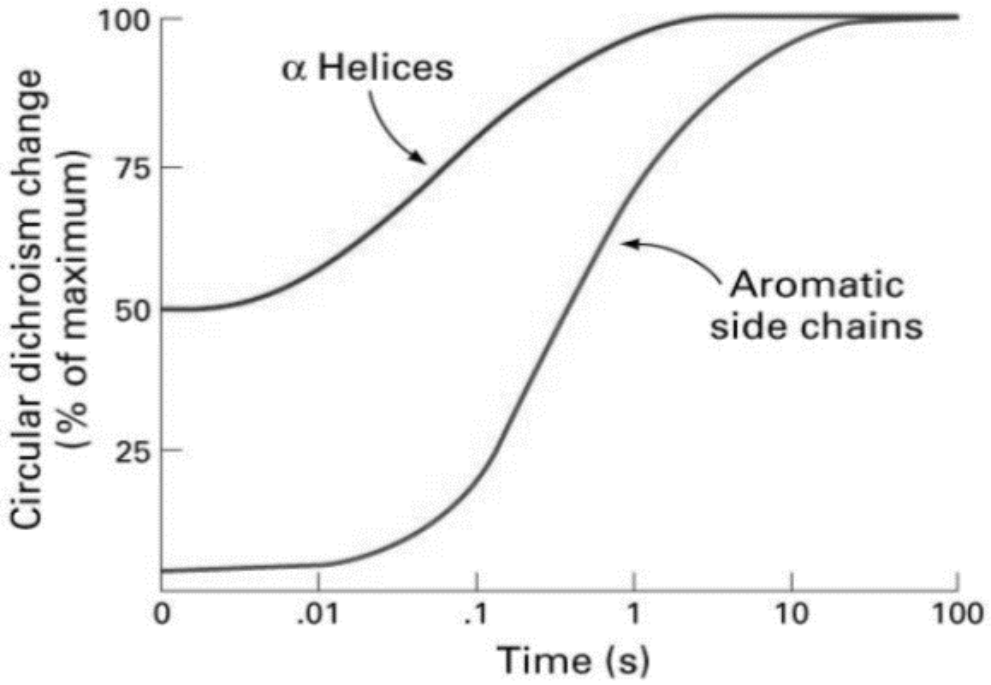
Lisa M. Miller, Megan W. Bourassa, Randy J. Smith. Biochimica et Biophysica Acta (BBA) - Biomembranes, Volume 1828, Issue 10, 2013, Pages 2339-2346, ISSN 0005-2736

Discussion

The figure on the right is the folding profile of protein X as monitored by circular dichroism. Among the three classic models of protein folding, which refolding model is:

(a)least suited for protein X? Explain your reasons. hydrophobic-collapse Model

(b)best suited for protein X? Explain your reasons. Nucleation Model



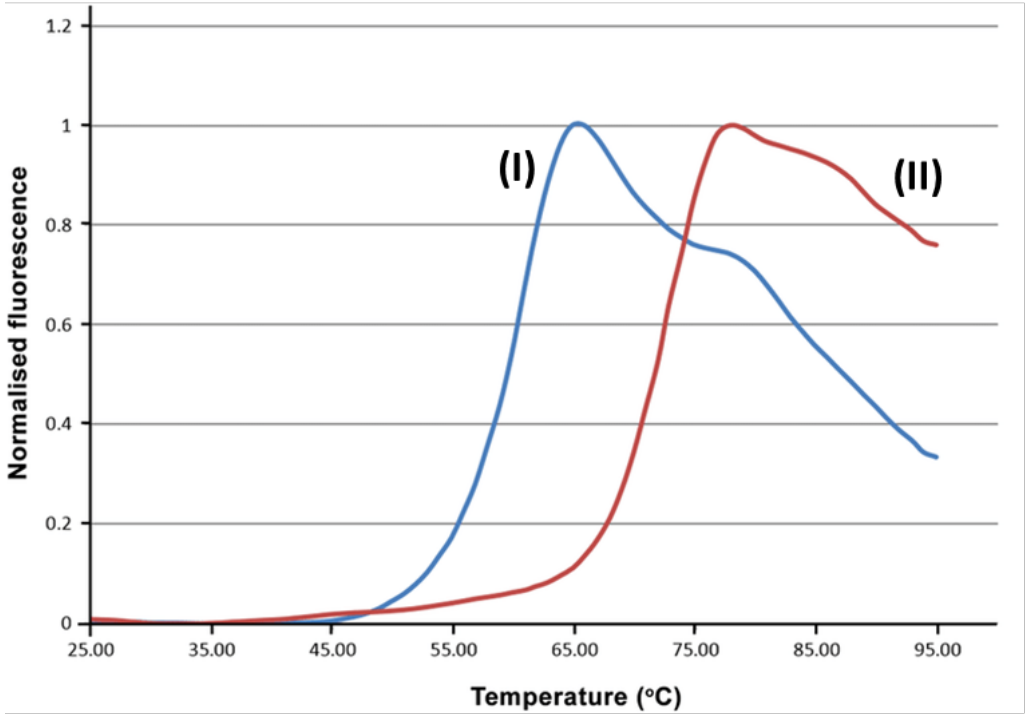
Practice

A thermal shift assay on an enzyme "Y" in the *absence and presence* of 1 mM of Inhibitor A, which has a known K_i value of 1 μM was performed. The result of the assay is shown in the figure.

Based on your knowledge on the thermal shift technique, which denaturation curve ((I) or (II)) in the figure is more likely to be the denaturation curve of:

(a) the apo-enzyme? 1

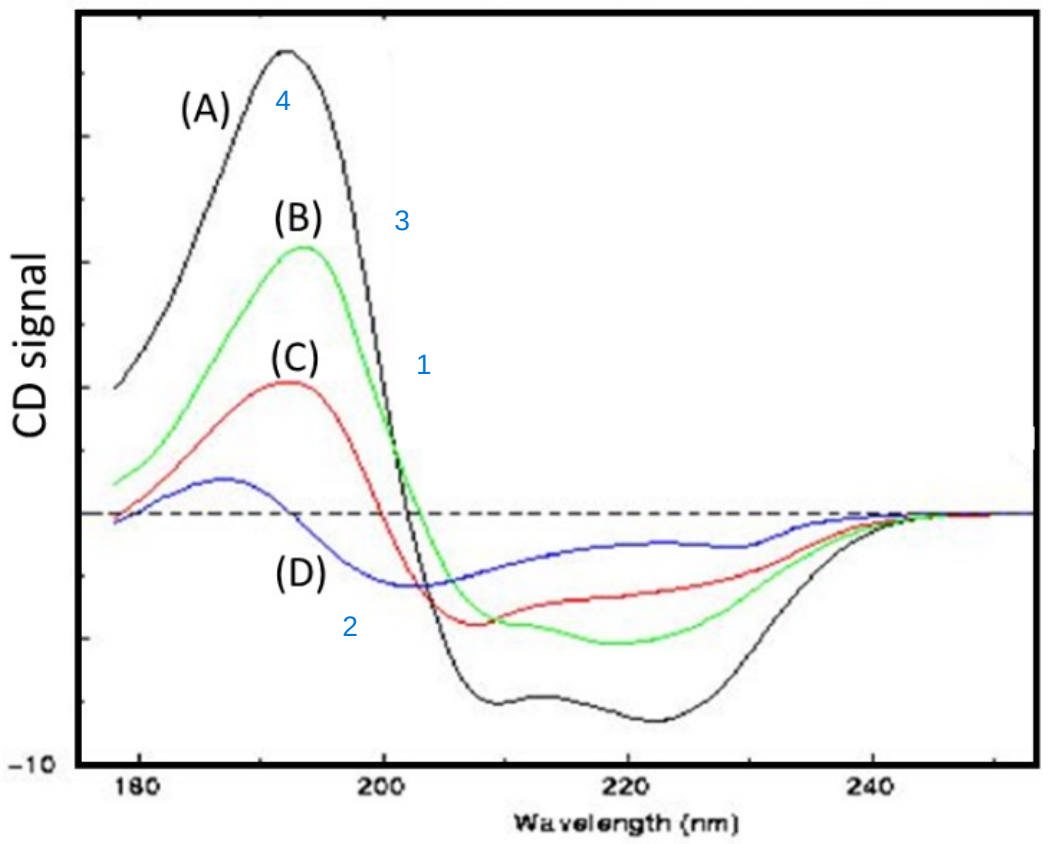
(b) enzyme Y + 1 mM Inhibitor A? 2



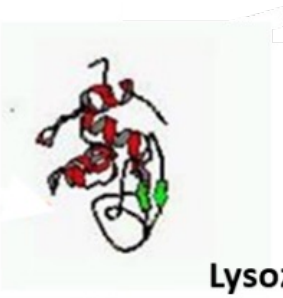
Practice

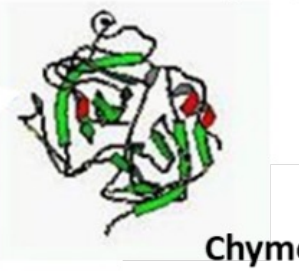
Which spectrum (A, B, C or D) is most likely to belong to:

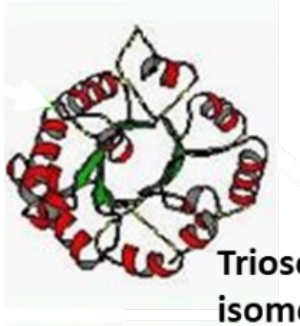
lysozyme? chymotrypsin? myoglobin?

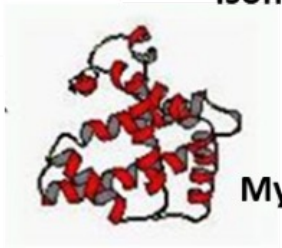


1. Lysozyme (40% helix, 20% β sheet)
2. Chymotrypsin (10% helix, 25% β sheet)
3. Triosephosphate isomerase (75% helix, 15% β sheet)
4. Myoglobin (96% helix)


Lysozyme


Chymotrypsin


Triosephosphate isomerase


Myoglobin